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THE RELATIVE LONGEVITY OF DIFFERENT STREPTOCOCCI AND POSSIBLE ERRORS IN THE ISOLATION AND DIFFERENTIATION OF STREPTOCOCCI *

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In the study of a large number of strains of streptococci I am impressed by the marked variation in the viability of the different strains. I have found that many types of streptococci may be stored for months on various media, while on the other hand, one frequently encounters strains that require fresh food material at short intervals.

The most favorable media for the preservation of the life of streptococci are those in which the organisms do not readily produce self-destructive substances. Such media are plain serum broth, blood agar, gelatin and others. It is very important that the media do not contain available carbohydrates as the acids formed by fermentation are detrimental to the life of these organisms.

EFFECTS OF ACID

Numerous investigators have observed the destructive effect of acids on streptococci. Koch and Pokschischewsky¹ used the fact that the streptococcus equi remained living in mannite serum broth after ten days as evidence that it had not attacked the carbohydrate. They also found that, by neutralizing at intervals dextrose and levulose cultures, acid continued to be produced up to the eighth day.

Wurtz and Mosny,² believing the rapid death of the pneumococcus in their medium was due to the acid produced, added calcium carbonate, and their cultures remained viable from one to six months.

Hiss³ used the same method to obtain material for immunization and agglutination. He used dextrose in his medium and added calcium carbonate to prevent the production of an excess of acid.

In another article I have discussed the relative longevity of different streptococci against the acids produced in fermentation. Koch and Pokschischewsky⁴ demonstrated that the streptococcus longus was more resistant to acid than the

* Received for publication May 29, 1914.

1. *Ztschr. f. Hyg. u. Infektionskrankh.*, 1913, 74, p. 1.

2. Quoted by Neufeld and Händel, *Handb. d. path. Mikroorg.*, 1912, 4, p. 522.

3. *Jour. Exper. Med.*, 1905, 7, p. 223.

4. *Ztschr. f. Hyg. u. Infektionskrankh.*, 1913, 74, p. 1.

streptococcus equi, as the former remained viable on dextrose broth longer than the latter. Von Lingelsheim⁵ has shown that the streptococcus brevis is more resistant than streptococcus longus to hydrochloric, sulphuric and oxalic acids.

EFFECT OF DESICCATION

The effect of drying of the media is important in considering longevity of streptococci. Besides the concentration of the salts and other constituents of the media we have to consider direct action of desiccation. Streptococci vary within wide limits in their power to survive a drying process. The majority of streptococci are very sensitive to desiccation. They usually die in a few days when dried on threads, glass and other substances. Hägler⁶ found that streptococci from erysipelas lived from fourteen to thirty-six days when dried on mull, and Kurth,⁷ that the streptococcus conglomeratus resisted drying for from five to six weeks. Germano⁸ noted that streptococci were very resistant if dried on dust, but different strains showed wide variations in their power to withstand this drying. Andrewes⁹ showed that certain strains, such as streptococcus equinus, will live for months when dried on garnets, while streptococcus pyogenes will only live for weeks under the same conditions.

The pneumococcus is generally much more sensitive to drying than the streptococci. Wood¹⁰ has thoroughly studied the viability of the pneumococcus after drying, and many other investigators have shown that its longevity is relatively short.

I have dried a strain of streptococcus fecalis from dextrose broth on cover-glasses and obtained growth after keeping in diffuse daylight for 174 days. The morphologic, cultural and fermentative characters remained unaltered (Holman).¹¹

The presence of streptococci in the air is an evidence of their resistance to a relatively high degree of drying. Chatin¹² isolated, from the air of rooms, different kinds of streptococci, some of which were highly pathogenic to rabbits, while others were non-pathogenic. Gordon¹³ found several different forms of streptococci in the outdoor air of London, streptococcus brevis being the most common type. Andrewes and Horder¹⁴ have shown that streptococcus equinus is the commonest type present in the air of London. It is also most abundant in horse dung. Rosenow¹⁵ obtained streptococci from the air of the hospital operating-room and the hospital wards. Winslow and Kligler,¹⁶ in a study of

5. Handb. d. path. Mikroorg., 1912, 4, p. 453.

6. Quoted by von Lingelsheim, *Ibid.*

7. *Ibid.*

8. *Ibid.*

9. *Lancet*, 1906, 2, p. 1415.

10. *Jour. Exper. Med.*, 1905, 7, p. 592.

11. *Am. Jour. Pub. Health*, 1913, 3, p. 1210.

12. These-Faculte de Medicine et de Pharmacie de Lyon, 1893, Serie 1, p. 812.

13. *Ann. Rep. Loc. Gov. Bd.*, 1902-3, p. 421.

14. *Lancet*, 1906, 171, p. 708.

15. *Amer. Jour. Obst.*, 1904, 50, p. 762.

16. *Am. Jour. Pub. Health*, 1912, 2, p. 663.

city dust, found in the air a variety of streptococci, chiefly of types characteristic of the human mouth and the human intestines. These authors conclude from their studies that certain streptococci are very resistant to drying.

RESISTANCE TO HEAT

Streptococci are also far more resistant to high temperatures than is commonly supposed. The destructive action of heat on bacteria in general is, after all, a relative one and we find that streptococci exhibit a marked variation in their ability to withstand high temperatures.

Van Lingelsheim¹⁷ has demonstrated that the streptococcus brevis is much more resistant to the action of heat than is the streptococcus longus. The former withstood 65 C. for twenty minutes, 70 for ten minutes, and 80 for five minutes, while the streptococcus longus succumbed after twenty minutes at 55 C., ten minutes at 60, and five minutes at 67.

In a later article the same author says that if a suspension of streptococcus longus is heated in normal salt solution to 60 C. for one or two hours it is not always sufficient to kill all the cocci. One hour at 70 or two hours at 65 is necessary for complete sterilization.

The character and composition of the fluid surrounding the cocci is of the greatest importance in determining the effects of heat. Albuminous fluids, such as milk, blood-serum and ascites fluid, have distinctly protective actions on the contained bacteria. In sterilizing such fluids for use in the preparation of media, this fact should be remembered.

In attempts to sterilize beef serum by heating to 56 C. for two hours on four successive days, I found that certain cocci remained living. On the other hand, these cocci grown in broth were promptly destroyed within two hours at 56 C. From a batch of milk which had been put through the Arnold sterilizer for twenty minutes on three successive days, we obtained a streptococcus mitis which had withstood the high temperature of flowing steam.

In order to further test this protective quality of serum, we used beef serum diluted one-half with water and sterilized by filtration. To this dilute serum we added a number of strains of streptococci, allowed the tubes to stand for ten days at the room temperature, and then heated the tubes to a temperature of 58 C. for twenty-three hours. Only one strain of streptococcus of four used survived the experiment. This strain had been recently isolated from the feces of a patient with dysentery and was of the streptococcus fecalis type.

A similar experiment, using ascites fluid and leaving the organisms in contact for only three days, showed that this same strain of streptococcus fecalis survived heating to 60 C. for six hours. No growths were obtained from any of the other thirteen streptococci used in this experiment. On a number of occasions, in attempts to isolate the bacillus Welchii from feces and other mixed materials by heating to 80 C. for 30 minutes, I have encountered in the cultures, streptococci, as well as the gas bacillus.

17. Handb. d. path. Mikroorg., 1912, 4, p. 453.

This work will be continued in an endeavor to determine the relationship between the relatively high resistance to heat of certain strains in albuminous fluids to the time of contact, the character of the fluid, the percentage of albumin, the number of organisms added, and other conditions that may have some bearing on the problem.

Ayers and Johnson,¹⁸ in a study of bacteria which survive pasteurization, have made some interesting observations on the resistance of bacteria in milk to high temperatures. These authors have shown that in heating milk to 62.8 C. there was little difference in the bacterial reduction produced by heating for one-half hour and by six hours' heating. The further study of their experiments, in heating samples of milk for a half hour at temperatures of 76.7, 82.2, 87.8, and 93.3 C., shows that their acid-forming group of bacteria, in a high percentage (80.91) survives the temperature of 76.7 C. The percentage is, however, greatly reduced at 82.2 C., and the higher temperatures. In a study of the cultures plated out from milk after pasteurization at 62.8 C. they noted that 89.39 per cent. of the acid-forming group of bacteria were cocci which produced no liquefaction of gelatin. The fermentation reactions of these and further cultures from two series, after heating to 60 C. and 65.6 C. for thirty minutes, would indicate that many of them were streptococci, although the authors do not state the morphologic arrangement of the cocci. This is all the more probable when we consider the frequency and high percentage of streptococci occurring in milk. By the typical lactic acid bacteria as shown in their table of reactions, the authors, no doubt, refer to the so-called streptococcus lacticus.

Frequent attempts have been made to separate the virulent streptococci from the non-virulent forms by the use of various materials, such as lecithin (Fromme,¹⁹ Machtle,²⁰ and others), but without success. Floyd and Wolbach,²¹ following the method of Churchman, have tested out the relative longevity of a series of streptococci in contact with gentian violet and conclude "that susceptibility to gentian violet may be a crude indication of the virulence of the streptococcus." Krumwiede and Pratt²² show that "the streptococcus-pneumococcus group differ from other gram-positive bacteria in their ability to grow in the presence of amounts of dye sufficient to inhibit the other species," and that the green streptococcus is not as sensitive as the hemolytic strain to the effect of certain dyes.

It is to be recognized that many of the virulent streptococci are more sensitive to a host of influences detrimental to the life of bacteria than are the more saprophytic types or the so-called non-virulent streptococci. This does not, however, hold in all cases and these methods of division of streptococci are being discarded for classification purposes.

LONGEVITY IN SYMBIOSIS

The wide distribution of streptococci and their constant association with other bacteria in the respiratory and intestinal tracts of man and animals in health and disease, as well as their presence in milk and

18. *Bull. Bur. Animal Indust.*, No. 161, 1913.

19. *Zentralbl. f. Gynäk.*, 1909, 33, p. 1217.

20. *Ibid.*, 1911, 35, p. 388.

21. *Jour. Med. Research*, 1914, 29, p. 493.

22. *Jour. Exper. Med.*, 1914, 19, p. 20.

other foodstuffs, make it of peculiar interest to learn something of their longevity in mixed cultures and in mixed infections. I have carried out a number of experiments with mixed cultures of different types of streptococci as well as with mixtures of streptococci and pneumococci and have found that they may live together for long periods of time if frequently transferred. Under unfavorable conditions, such as drying, etc., one or the other type disappears.

STREPTOCOCCI WITH STREPTOCOCCI

In my first experiment I used a strain of streptococcus pyogenes (570) from the pus of meningitis, and a strain of streptococcus fecalis (224) from an old empyema. These cultures were thoroughly mixed on a blood agar slant. Daily transfers were made to fresh blood agar slants and careful notes were kept each day of the appearance of the culture. Blood agar plates were made from time to time, colonies picked, and the freshly isolated cultures were tested for their fermentative reactions. These remained unaltered and distinctive. In this mixed culture hemolysis was the predominant feature up to the fifteenth transfer. On this transfer the blood, in general, was turned green, and with only two areas showing hemolysis. Further transfers showed the gradual return of the general picture of hemolysis, alterations were noted, a few green colonies appearing here and there on the slant at different intervals. On the twenty-ninth transfer there appeared numerous green colonies, but for the next ten transfers only hemolysis was to be seen. In plating on the surface of blood agar plates from these mixed cultures, two methods were used: (1) plates were smeared directly from the blood agar slants; and (2) plates were prepared after a preliminary growth in serum broth.

Several interesting observations were made. Direct plating from blood agar to blood agar usually showed a preponderance of hemolytic colonies and it was difficult at times to find isolated green colonies. Moreover, in picking from plates, showing both green and hemolytic colonies, the transfers from green colonies always proved to be pure, while transfers from the hemolytic colonies were sometimes mixed with the green strain. This is not to be wondered at, when we consider that the two organisms affect the blood in different degrees, one altering the hemoglobin and storing it in its colonies, the other bringing about hemolysis. The streptococcus with its more destructive action on red blood-cells will overshadow the character of the other type when the two are growing together. Moreover, the colonies of streptococcus fecalis are much smaller than those of the streptococcus pyogenes and were often observed within the hemolytic zone and in contact with the pyogenes colonies. The second method of plating, by growing in serum broth before smearing on the plates, allows the cocci to become well separated and the colonies developing are found to be pure. The

number of green colonies developing by this method is also greater, and their presence in the mixture is more easily determined.

STREPTOCOCCI WITH PNEUMOCOCCI

Parallel experiments were made with a strain of pneumococcus (556) obtained from a pneumonic lung at autopsy, and a strain of streptococcus fecalis (369) from feces of a patient with chronic constipation. This strain of streptococcus fecalis produced vegetations on the mitral valve of a rabbit after intravenous injection. The cultures were mixed as in the above experiment and daily transfers of the mixture were made. The pneumococcus colony is larger and more watery than that of the streptococcus fecalis, which is very small and dry. The results show that these two types grow together for long periods and can be reisolated in pure cultures. I encountered the same difficulty as previously noted in isolating colonies on the blood agar plates made directly from the blood agar slants. Transfers from the small green colonies proved to be pure, but those from the larger watery colonies quite frequently showed a mixture. Preliminary growth in serum broth eliminated this difficulty. The growth of the mixture gave the moist appearance of the pneumococcus culture up to the sixteenth transfer and from then until the thirty-ninth showed a more or less regular increase in the dry dotted characteristics of the streptococcus colonies, while here and there a more prominent colony was seen, supposedly of the pneumococcus. The colonies reisolated throughout the experiment were always tested for their fermentative reactions and never showed any alteration from the original strains.

The mixture, after being kept in the incubator for different lengths of time, was tested as a mixture in the carbohydrate serum broth media and usually gave the combined fermentation reactions of both strains. However, from some of the older mixed cultures only the reactions of the streptococcus fecalis were noted.

The pneumococcus remained living up to the last transfer, but it was often impossible to reisolate it from the older cultures. The ability to separate it from the mixture varied apparently with the condition of drying of the medium.

Eight dextrose serum broth tubes were inoculated from the fifth blood agar transfer of the mixture. These were grown for three days at 37 C. Blood agar plates were then made from the dextrose serum broth, but none showed any growth of the pneumococcus. Eight of the largest colonies were picked but they all proved to be the streptococcus fecalis. The high acid production in this medium, due to fermentation, would probably account for the suppression of the pneumococcus.

The growth from these eight tubes was separated by centrifugation, suspended in salt solution, divided equally, and injected into the peritoneal cavity of two guinea-pigs. After forty-eight hours, the guinea-pigs were killed, plates were made from the hearts' blood and 2 to 3 c.c. of blood injected directly into fresh guinea-pigs. The results are shown in Table 1. The pneumococcus was isolated in pure culture. A streptococcus mitis was isolated from the heart's blood of one animal. This result is discussed in a later part of this paper. All the colonies on the blood agar plates from the heart's blood were picked, and the cultures studied for their fermentative reactions. The streptococcus fecalis was not recovered.

The results of these experiments allow us to conclude that the relative longevity of streptococcus fecalis is greater on artificial media

than that of the pneumococcus, and that in the animal body the invasive power of the pneumococcus is greater than that of the streptococcus, or expressed in a different way, the streptococcus predominates on culture media while the pneumococcus gains the ascendancy in the animal body.

SYMBIOSIS WITH OTHER BACTERIA

The symbiotic relationships of the streptococci with other types of bacteria are also of great interest. In the intestinal and upper respiratory tract the streptococci are practically always present, living in closest contact with a host of other bacteria. Cultures from the peritoneal cavity following perforation of the intestines demonstrate that hemolytic streptococci are present in abundance, although cultures from the feces usually show that green-producing streptococci are in the greater numbers. With the bacillus diphtheriae, symbiosis is frequent and important. In obtaining pure cultures of the anaerobes and many other bacteria, streptococci are often difficult to eliminate. There are indeed a few organisms with which one or the other of the streptococci will not live (Gotschlich,²³ Rettger²⁴).

Working with a strain of subtilis bacillus, which contaminated mixed culture plates containing pneumococcus and streptococcus, I was able to show that the closest symbiotic relationship can exist for long periods. The colony of bacillus subtilis, which had developed on the more thickly seeded part of the plate, was transferred to a plain agar slant. After 24 hours there was a typical growth of an apparently pure culture of bacillus subtilis. A transfer was made to serum broth and after 24 hours' incubation, a blood agar plate was smeared with the growth. This plate showed the presence of streptococcus, pneumococcus and bacillus subtilis. The small colonies of the cocci were growing close to the subtilis growth, some touching the edge, others apparently within the subtilis colonies, while many were free, scattered in the vicinity. From all three types of colonies, transfers were made and cultures tested out on the various media. The pneumococcus and streptococcus were both recovered in pure culture. From some of the subtilis transfers streptococci were again isolated by plating through serum broth. After ten days in the incubator the mixture on the plain agar was again plated through serum broth and the streptococcus fecalis recovered. The pneumococcus had apparently disappeared. Seven transfers from plain agar to plain agar were made at various intervals and the streptococcus was isolated from each fresh culture of what appeared to be a pure growth of bacillus subtilis. The last plain agar transfer was made forty-one days after the contamination of the blood agar plate. From the second plain agar transfer, after it had been in the incubator for thirty-nine days, a blood agar plate was made through serum broth and the streptococcus was recovered. Colonies were picked from all plates and tested on the carbohydrate and other media. No alteration from the reactions of the original strains was noted.

A hemolytic streptococcus (*Streptococcus XII*), kindly sent by Dr. Rosenow, was used to test the effect of symbiosis with the bacillus subtilis. Four different strains of the bacillus subtilis group were used. Cross plates were made on blood agar. From the heavy growth of the bacilli, transfers were made to serum broths and from these, after incubation, blood agar plates were prepared. The hemolytic colonies on these plates were unaltered and were to be seen in closest contact with the bacillary colonies. From the serum broths plain agar slants were seeded and apparently pure cultures of the bacilli were demon-

23. Handb. d. path. Mikroorg., 1912, 1, p. 148.

24. Jour. Infect. Dis., 1905, 2, p. 562.

strated. After three days in the incubator, blood agar plates were again made through serum broth. The hemolytic colonies were unchanged on the third, while no growth of the streptococcus could be seen on the fourth. After nineteen days in the incubator on plain agar, blood agar plates from serum broth showed streptococci on only one of the plates. The hemolytic colonies on this plate were, however, typical. No green colonies were seen at any time in this series of experiments. The hemolytic colonies were often in closest contact with those of the bacilli.

From these experiments it would seem that the hemolytic strain of the streptococcus dies out more quickly than the green-producing type.

SECONDARY AND MIXED INFECTIONS

Symbiosis with other bacteria is a well-recognized method of raising the virulence of streptococci and pneumococci (Gotschlich). The study of mixed and secondary infections in human diseases demonstrates that the streptococci and pneumococci play an overwhelming rôle in these conditions. There are certain facts that must be borne in mind. The streptococci and the pneumococci enter the blood under many conditions of lowered resistance. The multiplicity of predisposing causes for pneumonia, the relative ease of streptococcal invasion in rheumatism, the frequency of secondary invasions in scarlet fever (Hektoen,²⁵ Klimenko,²⁶ Anthony²⁷), typhoid fever (Wassermann and Keyser²⁸), tuberculosis (Panichia,²⁹ Brown, Heise and Petroff³⁰), and other conditions of lowered resistance, the invasion in the dead body (Gwyn and Harris,³¹ Cannon,³² White³³) all bear out this statement. Moreover, it is not uncommon to find the pneumococcus as the cause of secondary conditions in streptococci infections, and vice versa.

SPONTANEOUS STREPTOCOCCUS INFECTIONS IN ANIMALS

What is true of man is also undoubtedly true for laboratory animals. Fatal epidemics caused by streptococci are not at all uncommon. A rapid review of the literature demonstrates this fact.

Charrin³⁴ found a streptococcus in a rabbit which had died from anthrax. Binaghi³⁵ isolated a capsulated streptococcus from a guinea-pig which died spontaneously. This was the first reported case of streptococcus mucosus infection. Boxmeyer³⁶ described a chronic infectious lymphadenitis of guinea-pigs which was caused by a streptococcus. Flexner³⁷ also found a streptococcus associated with a similar disease in guinea-pigs. Lamar³⁸ studied sixteen strains of this streptococcus which would indicate that the epidemic was a considerable one. Wittneben³⁹ studied an epizootic of guinea-pigs. Twenty-four died and a septicemia with pneumonia, pleuritis and pericarditis was found at autopsy. Streptococcus lanceolatus was isolated from these cases and was differentiated from the human strains of pneumococcus only by its easily stained capsule and its marked coagulation of milk. Weber⁴⁰ reports an epidemic of pneumonia

25. *Jour. Amer. Med. Assn.*, 1903, 41, p. 405.

26. *Centralbl. f. Bakteriolog.*, Abt. 1, Orig., 1912, 65, p. 45.

27. *Jour. Infect. Dis.*, 1909, 6, p. 332.

28. *Handb. d. path. Mikrobiolog.*, 1912, 1, p. 645.

29. Baumgarten's *Jahresbericht*, 1908, 24, p. 145.

30. *Trans. Nat. Assn. for Study and Prev. Tuberc.*, 1913, 9, p. 344.

31. *Jour. Infect. Dis.*, 1905, 2, p. 514.

32. *Die Bakteriologie des Blutes bei Infektionskrankheiten*, Jena, 1905.

33. *Jour. Exper. Med.*, 1899, 4, p. 425.

34. Quoted by LeGros, *Monographie des Streptocoques*, Paris, 1902.

35. Quoted by Shotmüller, *München. med. Wchnschr.*, 1903, 50, p. 849.

36. *Jour. Infect. Dis.*, 1907, 4, p. 657.

37. Quoted by Lamar, *Jour. Exper. Med.*, 1909, 11, p. 152.

38. *Jour. Exper. Med.*, 1909, 11, p. 152.

39. Baumgarten's *Jahresbericht*, 1907, 23, p. 149.

40. *Inaug. Dis.*, Munich, 1901.

among guinea-pigs. One-half of their rabbits died. At a later period the guinea-pigs became affected and thirty from a stock of fifty died. Streptococci were isolated from all thirty cases. Stephansky⁴¹ described a pneumococcus epidemic among guinea-pigs. The author isolated the pneumococcus from fourteen guinea-pigs, showing at autopsy a purulent and fibrinous inflammation of the lungs.

Selter,⁴² in his careful study of natural pneumococcus infection in laboratory animals, reported the isolation of pneumococcus lanceolatus from four guinea-pigs, one inoculated fourteen days before death with a pseudodiphtheria culture, another four days before, with killed tubercle bacilli, and a third after an inoculation two days before with a strain of bacillus dysenteriae. The fourth died spontaneously. Four rabbits also died with coccidiosis and pure cultures of pneumococcus were obtained from the cysts. The following winter another outbreak occurred in which one rabbit succumbed on the fourth day following inoculation with actinomyces, and five guinea-pigs died spontaneously or after the injection of cultures of different organisms. From these six animals pneumococci were also isolated. Lantz⁴³ speaks of sudden changes of temperature and improper ventilation as common causes of pneumonia in guinea-pigs. It is an extremely fatal disease in these animals. Salomon,⁴⁴ in his study of carbohydrate fermentation by the streptococci, includes in his list three strains of pneumococcus from an epidemic in guinea-pigs. Kutschera⁴⁵ found a streptococcus in an epidemic among white mice. Weil⁴⁶ in his investigations used, among other streptococci, a strain obtained from an epidemic in mice. Wherry⁴⁷ reports the finding of streptococci in two white rats. Andrewes and Horder⁴⁸ recovered from a mouse, inoculated with a pneumococcus, a gelatin-liquefying strain of streptococcus. These authors emphasize the liability of error in the use of animal passage for experiments on the constancy of the fermentation tests. Lamar⁴⁹ studied streptococci from three cases of fatal septicemia in monkeys, and pointed out the great importance of first understanding fully the disease of the laboratory animals.

In my experience, streptococci of various types have been obtained from guinea-pigs injected with other bacteria, and from guinea-pigs which died spontaneously. In Tables 1 and 2 the results of my experiments are given in condensed form. From these examples, as well as from the number of reports cited from the literature, it can reasonably be inferred that an invasion of the animal body by the streptococcus-pneumococcus group occurs under natural conditions, as well as from the injection of cultures of various bacteria. The strain of streptococcus mitis recovered from the heart's blood of Guinea-pig 6 was not, I believe, either a transformed pneumococcus or an altered streptococcus fecalis any more than the streptococci from the heart's blood of Guinea-pig 8, and those from the peritoneal cavity of Guinea-pigs 9 and 13 were derived from the dead or living cultures of the

41. Quoted by Selter, *Ztschr. f. Hyg. u. Infektionskrankh.*, 1906, 54, p. 347.

42. *Ibid.*

43. *Farmers' Bull. from U. S. Dept. Agric.*, No. 525.

44. *Inaug. Dis.*, Kiel, 1901, 8.

45. Baumgarten's *Jahresbericht*, 1908, 24, p. 136.

46. *Ztschr. f. Hyg. u. Infektionskrankh.*, 1911, 68, p. 346.

47. *Jour. Infect. Dis.*, 1908, 5, p. 515.

48. *Lancet*, 1906, 171, p. 708.

49. *Jour. Exper. Med.*, 1909, 11, p. 152.

TABLE 1
STREPTOCOCCI RECOVERED FROM INOCULATED GUINEA-PIGS

No.	Intraperitoneal Injection of	Result	Characteristics of Isolated Streptococci					
			Chains	Hemolysis	Lactose Serum Broth	Mannite Serum Broth	Salicin Serum Broth	Inulin Serum Broth
1	5 c.c. serum broth cultures of streptococcus from vagina†	Death after four days; streptococci isolated from peritoneum	Long	Green	+	+	+	+
2	5 c.c. serum broth culture from Guinea-pig 1	Death after 3 days; streptococci from peritoneum	Long	Green	+	+	+	+
3	Mixed culture of pneumococcus and streptococcus fecalis	Killed after 48 hours; cultures sterile			..	..	..	..
4	Same as No. 3	Killed after 48 hours; pneumococci from blood	—	Green	+	—	+	+
5	3 c.c. of blood from No. 4	Killed after 48 hours; pneumococci from blood	—	Green	+	—	+	+
6	3 c.c. of blood from No. 5	Killed after 48 hours; streptococci from blood	Medium	—	+	—	+	—
7	2 c.c. of blood from No. 5.	Killed after 48 hours; pneumococci from blood	—	Green	+	—	+	+
8	2 agar slants of colon bacilli	Died in 15 hours; colon bacilli and streptococci from blood	Short Long Short Medium	— — — —	— — — —	— — — —	+++ ++	++ ++
9	3 agar slants of colon bacilli heated 60 degrees for 1 hour	Killed in 48 hours; streptococci from peritoneum			..	..	..	..
10	20 c.c. dextrose serum broth culture of streptococcus 659	Killed in 48 hours; cultures sterile	Medium*	—	+	+	+	+
11	50 c.c. dextrose serum broth culture of streptococcus from guinea-pig intestine†	Killed in 48 hours; blood peritoneum; streptococci	Short**	—	—	+	+	+
12	Agar slant of colon bacillus	Killed in 16 hours; no growth			..	..	..	..
13	Peritoneal fluid from No. 12	Dead in 6½ hours; streptococci from peritoneum	Long	—	+	+	+	+

* From blood. ** From both blood and peritoneum.
† Hemolysis positive; lactose and salicin positive; mannite and inulin negative.
‡ Hemolysis negative; mannite and salicin positive; lactose and inulin negative.

TABLE 2
STREPTOCOCCI FROM GUINEA-PIGS DYING SPONTANEOUSLY*

No.	Anatomical Changes	Cultures	Characteristics of Isolated Streptococci					
			Chains	Hemolysis	Lactose Serum Broth	Mannite Serum Broth	Salicin Serum Broth	Inulin Serum Broth
1	Normal	Streptococci from cervical gland	Medium	+	—	—	+	—
2	Normal	No streptococci	—	—	—	—	—	—
3	Normal	Sterile	—	—	—	—	—	—
4	Pneumonia, pleuritis, congestion of small intestine	Streptococci (Peritoneum, Pleura, Blood)	Short†	—	+	+	+	—
5	Pneumonia	Streptococci from pleura and blood	Long†	+	+	—	+	—
6	Pneumonia	Streptococci from pleura and blood	Long	+	+	—	+	—
7	Pneumonia, pleuritis	Streptococci from pleura and blood	Long	+	+	—	+	—
8	Pregnancy	Pseudodiphtheria bacilli from blood	..	..	+	+	+	+

* These guinea-pigs were those dying spontaneously from a stock of about 700.

† From peritoneum.

‡ From pleura and blood.

TABLE 3
CHARACTERISTICS OF STREPTOCOCCI OBTAINED FROM GUINEA-PIGS

Source	Chains	Hemolysis	Lactose Serum Broth	Mannite Serum Broth	Salicin Serum Broth	Inulin Serum Broth	Notes
Intestinal Contents	Medium	—	—	+	+	—	See Nos. 1 and 2, Table 1.
Intestinal Contents	Medium	—	+	+	+	+	See Nos. 9, 11, 13, Table 1, and No. 4, Table 2.
Intestinal Contents	Medium	—	+	+	+	—	See No. 8, Table 1.
Intestinal Contents	Medium	—	+	+	+	+	
Throat	Short	—	—	—	+	+	
Throat	Short	—	+	+	+	+	
Throat	Long	—	+	+	+	+	

bacillus coli. In 1910, I recovered from the peritoneal cavity of a guinea-pig injected with a hemolytic streptococcus from the vagina, a non-hemolytic streptococcus which fermented inulin. I concluded that this organism had come from the animal.

Members of the streptococcus-pneumococcus group are found regularly in the intestinal and upper respiratory tracts of most animals (Selter,⁵⁰ De Gasperi,⁵¹ and others). I have isolated members of this group from the intestines and upper respiratory tract of a number of normal guinea-pigs. The cultural characteristics of these streptococci are shown in Table 3.

Many investigators have demonstrated by experiments the comparative ease with which organisms pass from the intestinal canal of animals into the bloodstream and the organs of the body. Loiseleur,⁵² Sacquepee and Loiseleur,⁵³ Basset and Carre,⁵⁴ Garnier and Simon⁵⁵ and others have shown that various methods of lowering the resistance by heat, cold, inanition, overexertion, injections of toxins, hypertonic solutions, dilute acids, and other conditions, lead to a bacteremia.

Adami⁵⁶ has shown that the tissues are potentially, but not actually, sterile. In animals fed with various cultures of bacteria these can be obtained, after a few hours, from the various organs of the body, for during the digestive process bacteria are absorbed along with the food, especially when much fat is present. Acute congestion of the mucosa of the intestines, however brought about, favors the passage of bacteria.

Simmonds⁵⁷ found that, after injecting rabbits with 500,000 to 2,500,000 killed streptococci, he had first a fall, then a rise in the opsonic index, and it is well known that injections of large numbers of bacteria regularly bring about a fall in the opsonic index. Young animals have been shown to be more susceptible to invasion than the full grown.

MUTATIONS

Buerger and Ryttenberg⁵⁸ believed that they had observed newly acquired properties in many of their strains of pneumococcus. It is interesting to note that in their first case the blood culture tested was taken two days before death and that this culture was readily converted from an atypical into a typical pneumococcus by passage through mice. A strain isolated from a metastatic abscess of this case showed the same general cultural characters. This latter strain could not be converted, and the former, after the lapse of one month, was also non-convertible. The authors state that the colonies from this blood culture showed "some ring forms" and that the morphology was "like degenerated pneumococci." I would conclude that the writers were dealing with a mixed infection in the blood culture, and a pure infection in the metastatic abscess. This explanation, based on the results of my experiments, as recorded in the earlier part of this paper, as well as on experience with mixed infections with pneumococcus, will serve to clear up the apparent confusion of the other cases cited by these authors.

Rosenow⁵⁹ in a recent communication claims that he has been able to transmute the pneumococcus to streptococci of various types, and vice versa.

50. *Ztschr. f. Hyg. u. Infektionskrankh.*, 1906, 54, p. 347.

51. *Centralbl. f. Bakteriolog.*, I, Orig., 1911, 57, p. 519.

52. *Baumgarten's Jahresbericht*, 1906, 22, p. 812.

53. *Ibid.*, 1907, 23, p. 703.

54. *Ibid.*, 1908, 24, p. 962.

55. *Ibid.*, 1909, 25, p. 969.

56. *Jour. Amer. Med. Assn.*, 1899, 32, p. 1509; *Br. Med. Jour.*, 1914, 1, p. 177.

57. *Jour. Infect. Dis.*, 1907, 4, p. 595.

58. *Ibid.*, p. 609.

59. *Ibid.*, 1914, 14, p. 1.

Schereschewsky⁶⁰ believes that the streptococcus pyogenes is closely related to the pneumococcus. He was able to bring about very marked morphologic changes and believed he was able to change one form into the other. Schereschewsky's results have not been accepted as indicating any fundamental alteration of these organisms.

Rosenow has gone much further and he is convinced that "the transformations of some of the strains are complete by every known test." He has not only altered the morphology, the formation of capsules, and the fermentative powers, but also the specific immunity response, and the more or less specific pathogenic powers. The results of my experience and those of numerous other workers would tend to throw doubt on many of his interpretations.

In the detailed description of some of his experiments I am able to follow some of the steps of his transmutations. In Strain 595 he had a hemolyzing culture which, after growing for over a year and showing no important change during this time, was transferred to blood agar slants and left in the incubator twenty-nine days until "the media were very dry." Transfers were now made to fresh blood agar, and after eight days at 37 C. the surface of a smeared blood agar plate showed two types of colonies, one hemolyzing, and the other not. This would appear to the ordinary observer to have been either from a survival of a non-hemolyzing strain, as shown in my experiments, or to have been a contamination. These non-hemolyzing colonies were first noted on November 15. On November 17, four colonies of each variety were plated on blood agar plates. One of the non-hemolyzing colonies, thus plated, showed on November 19 two types of colonies, adherent and non-adherent. On this same date, before these two types of colonies had been studied further, Rosenow injected four small rabbits (600-750 gm.) with the growth from 20 to 60 c.c. of ascites dextrose broth culture of this streptococcus viridans strain. One colony from the blood of one of these rabbits was used for the further transmutation into pneumococcus. On November 21, transfers were made from the culture of an adherent green colony to blood agar and Loeffler's serum. After seventy-seven days the growth on Loeffler's serum yielded both hemolyzing and non-hemolyzing colonies. Is this evidence of reversion or the indication of a mixed culture? The author does not give the fermentative and other characteristics of these various strains when first isolated as a streptococcus viridans, when recovered from the first injected rabbit, nor after the first two guinea-pig injections. The transformed pneumococcus, after being subcultured for five months, yielded a hemolytic strain which showed fermentation of mannite, a reaction not shown by either the original hemolytic strain or the pneumococcus. In such an important question as the conversion of such well differentiated organisms as the pneumococcus and the hemolytic streptococci, full and complete cultural and other characters should be given at every step in the alteration. That the organisms at the beginning and at the end of the experiment are quite different in every important character we have no doubt, but if transmutation has really occurred, we must know when the changes took place.

The transmutations by growth with the bacillus subtilis we have been unable to confirm, although we used a hemolytic strain (XII) kindly sent me by Dr. Rosenow, and cultured it in intimate contact with four strains of the subtilis bacillus. We were, however, able to show that a strain of streptococcus fecalis grew with the bacillus subtilis for long periods of time, and could be best recovered by plating on blood agar after first growing the mixture in serum broth. Strain XII as a hemolyzer, had been converted by Rosenow into strep-

60. *Centralbl. f. Bakteriol.*, Abt. 1, Orig., 1909, 49, p. 72.

tococcus viridans by growth in symbiosis with the bacillus subtilis. The full characteristics of the three varieties of colonies, obtained from this strain after growth on ascites-dextrose agar, are not given. It is to be noted that ascites fluid seemed to be necessary for the alteration into the rheumatic types of streptococci with this and other strains discussed under "the effect of growth in a high oxygen pressure." The author used in his media, in the study of the etiology of acute rheumatism, ascites fluid which yielded uniform and frequent positive results. The very uniformity is striking. In one case he obtained two colonies per 20 c.c. from the right knee, two colonies per 12 c.c. from the left knee, three colonies per 2 c.c. from the wrist, and ten colonies per 0.05 c.c. of bloody fluid from a red, tender, swollen area over the lower end of the ulna. Is it possible that the ascites fluid was not sterile? The method of heating to 60 C. for twenty-four hours to obtain sterile fluid is not as certain as careful filtration. In testing such a fluid for the presence of a few organisms, large quantities must be employed. Recently I obtained from a peritoneal swab, taken at operation, a strain of streptococcus which showed a marked tendency to anaerobism. It grew best in the depths of carbohydrate serum agar media and did not develop on the surface till after several transfers. It did grow, however, in very minute colonies on fresh blood agar slants. Unfortunately, the resistance to heat of this streptococcus was not tested. Such an organism would be readily overlooked in testing the sterility of human fluid for use in culture media.

Strain 734 was claimed to have been changed by aerobic culture in distilled water from a non-mannite green-producer into a hemolyzer and mannite fermenter. Its virulence was also distinctly greater than before. The raising of virulence by growth in water is contrary to the experience of all workers in water bacteriology.

From my experiments with guinea-pigs, and the results of the other investigators cited, I would be unwilling to believe that the recovery of green streptococci from a joint, or the pericardium, or other cavities of animals injected with large doses of a hemolytic streptococcus, is sufficient evidence to conclude that the recovered organism is the same as that injected.

The altered form of Strain 734, which was subsequently changed to pneumococcus, was derived from the pericardium of a rabbit. Strain 736 "in the joint of one rabbit, in its second passage, lost the power to hemolyze and produced green colonies instead." When Strain B, a "pure line," which had acquired the power to hemolyze blood agar, was recovered from the joint of one rabbit it was found mixed with green streptococci. This latter strain was then transformed into pneumococcus. Strain R 51A was originally a pneumococcus, but had acquired the power on dried blood agar to hemolyze blood for the first time in over ten years. This strain was passed through sixteen rabbits. The joint culture from the sixteenth animal showed both green and hemolyzing colonies. A hemolytic streptococcus, derived from a pneumococcus, was injected into a rabbit, which five days later was found dead. Joint fluids yielded three green and no hemolyzing colonies.

The number of guinea-pigs used, before the transmutation of strains of streptococcus viridans into pneumococcus took place, varied greatly. My interpretation of this fact is that the invasion from the animal varied with the different animals, or the invading organisms were not recovered until they had gained a numerical ascendancy.

Andrewes,⁶¹ in an excellent paper, has discussed the nature and degree of specific differences amongst bacteria, and his points are well

61. *Lancet*, 1906, 2, p. 1415.

worth consideration for maintaining a conservative view of the questions of differentiation of bacteria.

CONCLUSIONS

Streptococci vary within wide limits in their longevity on artificial media. They also show wide differences in their sensitiveness to the effects of desiccation.

The presence of acid, produced in media containing carbohydrates, is very detrimental to the life of streptococci. The widest variation is exhibited among different strains to this effect of acid.

Many strains of streptococci are resistant to high temperatures over prolonged periods of time, more especially when protected by albuminous fluid as in milk, beef serum and ascites fluid.

Streptococci of all forms live in closest symbiotic relationship with many other bacteria.

Hemolytic streptococci, growing on blood agar in mixed culture with a green-producing form, show an apparent predominance, and pneumococcus and streptococcus, in mixed cultures, show that the growth of the pneumococcus may be overshadowed by that of the streptococcus.

Plating on blood-agar after a preliminary growth in serum broth is a more certain method for obtaining pure cultures than direct plating from blood-agar to blood-agar.

The injection of known mixed cultures of pneumococcus and streptococcus, which culturally appear to be pure streptococcus, shows that the more invasive form, the pneumococcus, survives.

In mixed cultures of pneumococcus and streptococcus *fecalis* the former disappears first, as the result of drying of the media.

Strains of green streptococci will live for long periods in mixed cultures with the bacillus subtilis, while the pneumococcus and hemolytic streptococci die out more quickly.

The growing of hemolytic streptococci with cultures of the bacillus subtilis for long periods has no effect in altering its hemolytic properties.

Streptococci and pneumococci invade the body under many and various predisposing conditions. They are also by far the most frequent secondary invaders.

These organisms spontaneously invade the bodies of our laboratory animals bringing about fatal epidemics. Various predisposing causes make these invasions possible.

The intestinal and upper respiratory tracts of guinea-pigs contain different forms of streptococci.

Injections of bacteria into guinea-pigs, especially young animals, bring about an invasion into the body cavities and the blood-stream of organisms, more particularly of various strains of streptococci derived from the normally infected regions of the animal.

Isolation from animals of streptococci, differing in important characteristics from those injected, cannot be taken as proof of a change in the characteristics of the injected streptococcus within the animal body, owing to the ease with which streptococci are able to enter the tissues from the intestines or respiratory tract of animals as well as man.

In attempts at transmutation all the characteristics of the organisms must be given at every stage of the experiment.